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EP 0 284 232 B1

Description

This invention relates to an assay for an analyte, and more particularly to a solid phase assay.

Assays for various analytes have been accomplished by a so-called solid phase assay. In a solid phase assay, a binder specific for at least the ligand to be determined (analyte) is supported on a solid support, whereby, in the assay it is not necessary to employ an additional agent for separating the bound and free phases formed in the assay.

In general, such solid supports have been in the form of tubes, solid particles, and in some cases, the solid phase has been in the form of a "dip-stick".

In a dip-stick solid phase assay, a binder may be supported on the dip-stick with the dip-stick, containing the binder, being dipped into an assay solution containing the analyte, and in general, such solution further contains a tracer. The presence and/or amount of tracer on the dip-stick is then employed as a measure of analyte (either a qualitative or quantitative measure of analyte).

The present invention is directed to providing an improved solid phase assay for determining analyte, and more particularly to a solid phase assay.

In accordance with one aspect of the present invention, there is provided an assay device for determining the presence or absence of an analyte in a liquid sample comprising:

a) a test strip having at least a first and second portion and being arranged on the strip in the same plane in a manner such that material can flow by capillary attraction from the first portion to the second portion;

b) said first portion having a tracer movably supported therein wherein said tracer comprises a ligand, specific for the analyte when the device is configured for a sandwich assay and is the analyte or analogue thereof when the device is configured for a competitive assay, conjugated to a non-soluble particulate marker and being the site for addition of the sample;

c) said second portion having immobilized therein a binder which is specific for the analyte when said device is configured for a sandwich assay and is specific for the analyte and ligand when said device is configured for a competitive assay; the binder being present in an amount such that tracer bound in such portion is visible.

The tracer is supported on the first portion of the solid support in a manner such that when wetted, the tracer is capable of being transported by capillarity to the second portion of the solid support, and thereafter, optionally, to a third portion of the solid support.

The binder which is supported on the second portion of the solid support is supported in a manner such that the binder remains immobile and is not transported by capillarity to the third portion of the solid support.

The third portion of the solid support may be a portion for detecting unbound tracer which has been transported by capillarity from the second portion to the third portion. The third portion may or may not include a substance supported thereon for detecting tracer. Alternatively, the third portion may function only to receive materials not bound in the second portion.

In accordance with the present invention, the amount of tracer which is immobilized in the second portion of the solid support by being bound either directly to the binder in the second portion (in a competitive assay format), or by being indirectly bound to the binder (tracer is bound to analyte which is bound to the binder in a sandwich assay format) is dependent upon the presence and/or amount of analyte in the sample.

In a preferred embodiment of the present invention, the solid support and the various components are produced and employed in a manner for determining analyte by a competitive assay format.

In a particularly preferred embodiment, as hereinafter explained in more detail, the particulate marker portion of the tracer is comprised of a sac or lipid vesicle (often referred to as a liposome).

In employing a preferred embodiment wherein the assay is a competitive assay, the tracer is supported on the solid support on the first portion thereof, and the first portion of the solid support is wetted with the sample containing analyte to be determined. Upon wetting of the solid support with the sample, both sample and tracer flow by capillarity into the second portion of the solid support which contains a binder specific for both the analyte and tracer, with the binder being immobilized on the second portion of the solid support. Depending upon the presence and/or amount of analyte in the sample portion, tracer becomes bound to the binder on the second portion of the solid support. The tracer which is not bound by the binder on the second portion may then flow by capillarity into a third portion of the solid support. A quantitative assay may be run by determining tracer which remains in the second portion of the solid support and comparing such detected amount of tracer in the second and third portion with a "standard curve" to determine the amount of analyte in the sample. Thus, in an assay the determination of tracer and/or analyte may be either qualitative or quantitative.

In a "yes or no" sandwich assay type format, th amount of tracer which is employed on the first portion of the solid support as well as the amount of binder on the second portion of the solid support are such that in the presence of a detectable amount of analyte, essentially no detectable tracer may flow into the third por-

tion of the solid support.

In a sandwich assay format, th amount of binder which is employed on the second portion of the solid support is an amount such that essentially all of the analyte which is suspected of being present in the sample is bound by the binder on the second portion.

The solid support is one which is capable of absorbing analyte from the sample, and which, when wetted, provides for flow of analyte and tracer by capillary attraction from the first portion, and to the second portion and optionally into the third portion of the solid support. In addition, the solid support is one which is capable of supporting tracer and the binder. As representative examples of suitable solid supports there may be mentioned: glass fiber, cellulose, nylon, crosslinked dextran, various chromatographic papers, nitrocellulose, etc. A particularly preferred material is nitrocellulose.

The solid support is preferably shaped in the form of a strip, with the successive portions being arranged on the strip in the same plane in a manner such that material can flow by capillary attraction from the first portion and through the second portion and, optionally, to the third portion. Although the preferred shape is in the form of a strip, any other of a wide variety of shapes or forms may be employed as long as the shape and form permits separate portions for performing the various functions, as hereinabove described.

The tracer employed in the assay, as hereinabove indicated, is comprised of a ligand portion and a particular marker conjugated to the ligand portion.

The ligand portion of the tracer is dependent upon the assay format. If the assay is a competitive assay, then the ligand portion of the tracer is either the analyte or an appropriate analogue thereof. An appropriate analogue means that the analogue of the ligand is also specifically bound by the binder for the analyte. If the assay format is a sandwich type of assay, then the ligand portion of the tracer is a ligand which is specifically bound by the analyte or by an antibody which is specifically bound by the analyte.

The binder which is employed in the assay is one which at least binds the analyte. As hereinabove indicated, if the assay format is a competitive type of assay format, then the binder also binds the ligand portion of the tracer.

As generally known in the art, if the analyte is an antigen or a hapten, then the binder may be either a naturally occurring binder or an antibody which is specific for the analyte (either a polyclonal and/or monoclonal antibody). If the analyte is an antibody, the binder may be either an antigen specific for the antibody or an antibody which specifically binds the antibody analyte.

The binder may be supported on the solid support in a manner which immobilizes the binder; e.g., adsorption, covalent coupling, etc. The procedures for immobilizing binders on a solid support are generally known in the art.

The tracer is supported on the first portion of the solid support in a manner such that when the first portion in a manner such that when the first portion is wetted the tracer flows by capillary action. Thus, for example, the tracer may be adsorbed on the first portion of the support.

In accordance with a particularly preferred embodiment of the present invention, in a competitive assay, the tracer is comprised of a ligand conjugated to a vesicle as particulate marker with the tracer being supported on the solid support. Applicant has found that it is possible to support such a tracer on a solid support of the type hereinabove described, and that such tracer will flow by capillarity when the solid support is wetted with a sample containing or suspected of containing an analyte.

The lipid vesicles (liposomes) which are employed may be prepared from a wide variety of lipids, including phospholipids, glycol lipids, and as representative examples there may be mentioned lecithin, spingomyelin, dipalmitoyl lecithin, distearoylphosphatidylcholine, etc. The amphiphilic lipids employed for producing liposomes generally have a hydrophilic group, such as a phosphato, carboxcyclic, sulfato, or amino group, and a hydrophobic group, such as saturated and unsaturated aliphatic hydrocarbons, and aliphatic hydrocarbon groups substituted by one or more aromatic or cycloaliphatic groups. The wall forming compounds for producing the liposomes may further include a steroid component such as cholesterol, cholestanol, and the like. The compounds for producing liposomes are generally known in the art, and no further details in this respect are deemed necessary for a complete understanding of the present invention.

The liposomes may be produced by procedures generally available in the art. For example, liposomes may be produced by a reverse phase evaporation technique wherein the compound or compounds used in producing liposomes are initially dissolved in an organic phase, followed by addition of an aqueous phase and forming of a homogeneous emulsion. After forming the emulsion, the organic solvent is evaporated to form a gel like material, and such gel may be converted to a liposome by agitation or dispersion in an aqueous media.

Procedures for producing liposomes are described, for example, in U.S. Patent No. 4,241,046; U.S. Patent No. 4,342,826 and PCT International Publication No. WO 80-01515.

If a material, such as a dye, is to be encapsulated in the liposome, such material may be encapsulated in the liposome by including the material in the aqueous solution in which the liposome is formed. Alternatively,

the material many be encapsulated into a previously formed empty liposome (without material to be encapsulated) by the procedure described in U.S. Patent No. 4539376.

The liposomes may also be produced by the procedures disclosed in U.S. Patent No. 4,522,803.

The material which is entrapped or encapsulated within the liposome (the material is within the aqueous compartment or within the membrane bilayer of the liposome) is a detectable marker, such as dyes.

The liposome is derivatized with a ligand for producing a tracer. The liposome may be derivatized with a ligand by procedures known in the art, such as covalent coupling, derivatization or activation, etc. In derivatizing the liposomes with a ligand, a compound or compounds used in forming the liposome may be derivatized with the ligand, prior to forming the liposome, or alternatively, the liposome may be derivatized with the ligand, subsequent to forming of the liposome. Procedures for derivatizing liposomes with ligands, and suitable coupling agents, and the like for preparing derivatized liposomes are known in the art, and no further details in this respect are deemed necessary for a complete understanding of the present invention.

In employing a preferred tracer in which the particulate marker portion thereof is comprised of a liposome for use in a competitive assay, the assay may be accomplished as hereinabove described with general reference to a variety of tracers, except that the tracer includes a liposome as the particulate marker portion of the tracer.

The particulate label may also, for example, be a metal or alloy (e.g. colloidal gold). The marker preferably included in the sac is a dye or some other material which is visible, without lysing of the sacs.

The tracer comprised of ligand and particulate marker may also be produced by labeling the ligand with an aqueous dispersion of a hydrophobic dye or pigment, or of polymer nuclei coated with such a dye or pigment. Such labels are described in more detail in U.S. Patent No. 4,373,932, which issued on February 15, 1983. The tracers produced in accordance with such patent may also be employed as tracers in the present invention.

As indicated in the aforesaid patent, the colored organic compounds which are used as labels are in the form of a hydrophobic sol, which hydrophobic organic dyes or pigments are insoluble in water or soluble only to a very limited extent.

The visible particulate label may be visible polymer particles, such as colored polystyrene particles, preferably of spherical shape.

The second portion of the assay device which includes the binder is formed of a material having a surface area capable of supporting the binder thereon in an amount such that tracer bound in such portion is visible. In general, the surface area is capable of supporting the binder in a concentration of at least 1 $\mu g/cm^2$, and most generally in a concentration of at least 10 $\mu g/cm^2$. A particularly preferred material is nitro-cellulose. Such materials and tracers are described in EP-A-0154749.

The invention will be further described with reference to the accompanying drawing, wherein:

The drawing is a schematic drawing of a dip-stick in accordance with the present invention.

Referring to the drawing, there is shown a strip including a first portion A on which a tracer is supported; a second portion B on which a binder is supported and a third portion D. As particularly shown, a portion C is between portions B and D to provide spacing between portions B and D.

In a competitive assay format, portion A of the strip 10 would be contacted with a sample containing analyte, whereby portion A would be wet with the sample. The tracer in portion A, as well as sample, is transported by capillarity to portion B, where tracer and analyte compete for binding sites on the binder. Unbound tracer and unbound analyte move by capillarity through portion C to portion D which would be blank, tracer being determined in portion B.

The product may be used as a dip stick. Alternatively, a sample may be applied to portion A. Accordingly, the product may be used in either a horizontal or vertical orientation.

The invention is applicable to detecting and/or measuring a wide variety of analytes, such as: drugs, including therapeutic drugs and drugs of abuse; hormones, vitamins, proteins, including antibodies of all classes, peptides; steroids; bacteria; fungi; viruses; parasites; components or products of bacteria, fungi, viruses, or parasites; allergens of all types; products or components of normal or malignant cells; etc. As particular examples, there may be mentioned T_4; T_3; digoxin; hcG; insulin; theophylline; leutinizing hormone; organisms causing or associated with various disease states, such as streptococcus pyrogenes (group A), Herpes Simplex I and II, cytomegalovirus, chlamydiae, rubella antibody, etc.

The invention will be further described with reference to the following example:

Example

Dipsticks were constructed by first coating 0.5 x 8 cm strips of polystyrene with Scotch(R) #969 adhesive transfer tape (3M, St. Paul Minnesota 55144). Zone B, consisting of a 0.5 x 0.5 cm square of 5 um-pore nitrocellulose (S&S, Keene, New Hampshire) was spotted with 3 ul of affinity purified rabbit anti-Group A Strepto-

coccus antigen and then blocked with 3% bovine serum albumin. After drying, it was applied to the taped side of the dipstick, approximately 1 cm from the bottom of the stick. A strip of filter paper 0.5 x 6.5 cm. (Whatman 3 mm) was applied just above and touching the nitrocellulose, at the positions indicat d by zon s C and D. Zone A, consisting of dry Sephadex G50 fine grade (Pharmacia) was then applied.

Detector liposomes packed with sulfo-rhodamine dye were prepared by the method outlined in O'Connell et al. (Clin. Chem. 31:1424 [1985]). They were covalently coupled to affinity purified rabbit anti-Group A Streptococcus antigen.

The detector liposomes were spotted (2 ul) onto Zone A, 0.5 cm from the bottom and air dried. The liposomes are in a 0.05 M Tris buffer, pH 6.8, containing 2% glycerol, 0.05% dimethyl sulfoxide, 20 mM EDTA.

Group A Streptococcus organisms were harvested from culture plates, washed with saline (0.9% NaCl), and adjusted to 1 x 10^9 organisms/ml. An aliquot (0.1 ml) containing 1 x 10^8 organisms was subjected to the micro nitrous acid extraction method for exposing the Group A carbohydrate antigen. This method consists of mixing 0.3 ml of 0.1 M HCl with 40 ul of 4M $NaNO_2$, adding this to the Streptococcus organisms and, after 3 minutes, neutralizing with 40 ul of 1M Tris base. To faciliate the extraction and the dipstick assay, the HCl and the subsequent diluting fluid contain 0.1% Tween-20 non-ionic detergent.

Using the extracted antigen, a dilution series was prepared ranging from 8 to 10^6 organisms/ml to 1.25 x 10^5 organisms/ml. Aliquots of these dilutions (0.5 ml) were placed in 12 x 75 mm test tubes and a dipstick placed into the fluid in each test tube. As the fluid containing extracted antigen wicks up the stick, it carries the liposome detector past the spot of capture antibody. In the presence of antigen, which binds to the capture antibody spot, some of the liposomes also bind, resulting in the appearance of a red spot in zone B. The remainder of the liposomes and antigen solution pass into zone D.

The assay can be "read" by observing the lowest concentration of organisms resulting in a red spot in zone B. The results of this example are given in the following table and indicate an end point of 5 x 10^5 organisms/ml, close to the sensitivity required for a direct throat swab diagnostic for Group A Streptococcus pharyngitis.

Group A Strep Antigen (organisms/ml) x 10^{-5}

80	40	20	10	5	2.5	1.25	0
+	+	+	+	+	-	-	-

(+) = positive indication of antigen (red spot)

(-) = negative indication of antigen (red spot)

The present invention is advantageous in that there is provided a product and process which may be easily employed for accomplishing an assay. The product and process do not require the addition of tracer in that tracer is included in the product. In addition, the product and process are capable of providing for a rapid assay.

Claims

1. An assay device for determining the presence or absence of an analyte in a liquid sample comprising:
   a) a test strip having at least a first and second portion and being arranged on the strip in the same plane in a manner such that material can flow from the first portion to the second portion;
   b) said first portion having a tracer movably supported therein wherein said tracer comprises a ligand, specific for the analyte when the device is configured for a sandwich assay and is the analyte or analogue thereof when the device is configured for a competitive assay, conjugated to a non-soluble particulate marker and being the site for addition of the sample;
   c) said second portion having immobilized therein a binder which is specific for the analyte when said device is configured for a sandwich assay and is specific for the analyte and ligand when said device is configured for a competitive assay; the binder being present in an amount such that tracer bound in such portion is visible.

2. A device as claimed in claim 1 wherein the detectable particulate marker is a coloured liposome or coloured polymeric bead.

3. A device as claimed in claim 2 wherein the particulate marker is a coloured polystyrene bead.

4. A device as claimed in any one of claims 1-3 wherein the analyte is an antibody and the ligand is an antigen.

5. A device as claimed in any one of claims 1 to 3 wherein the analyte is an antigen and the ligand is an antibody.

6. A device as claimed in any one of the preceding claims which includes a third portion downstream of the second portion and being in capillary flow communication with said second portion.

7. A device as claimed in anyone of the preceding claims wherein the second portion of said test strip comprises nitrocellulose.

8. A no-wash, one-step method for determining the presence or absence of an analyte in a liquid sample consisting of the steps of:
   a) adding a liquid sample to the first portion of the device as claimed in any one of the preceding claims;
   b) allowing sufficient time for the liquid to mix with the tracer and for the tracer-analyte mixture to flow to the second portion of the device of claim 1; and
   c) reading the result by detecting the presence or absence of the visible particulate detectable marker in the second portion.

Patentansprüche

1. Testeinrichtung zur Feststellung der Gegenwart oder Abwesenheit eines Analyten in einer Flüssigkeitsprobe, mit:
   a) einem Teststreifen mit mindestens einem ersten und einem zweiten Abschnitt, die auf dem Streifen in der gleichen Ebene so angeordnet sind, daß Material vom ersten zum zweiten Abschnitt fließen kann;
   b) wobei der erste Abschnitt einen beweglich eingelagerten Tracer mit einem Liganden aufweist, der für den Analyten spezifisch ist, wenn die Einrichtung für einen Sandwich-Test aufgebaut ist, und welcher der Analyt selbst oder eine zum Analyten analoge Substanz ist, wenn die Einrichtung für einen Kompetitivtest aufgebaut ist, und wobei der Abschnitt mit einem unlöslichen, aus Feststoffteilchen bestehenden Markierungsstoff zusammengesetzt ist und den Ort darstellt, wo die Probe zugesetzt wird;
   c) wobei der zweite Abschnitt ein darin immobilisiertes Bindemittel aufweist, das für den Analyten spezifisch ist, wenn die Einrichtung für einen Sandwich-Test aufgebaut ist, und das für den Analyten und den Liganden spezifisch ist, wenn die Einrichtung für einen Kompetitivtest aufgebaut ist; wobei das Bindemittel in einem solchen Anteil vorhanden ist, daß der in diesem Abschnitt gebundene Tracer sichtbar ist.

2. Einrichtung nach Anspruch 1, wobei der nachweisbare, aus festen Teilchen bestehende Markierungsstoff ein farbiges Liposom oder ein farbiges Polymerkügelchen ist.

3. Einrichtung nach Anspruch 2, wobei der aus festen Teilchen bestehende Markierungsstoff ein farbiges Polystyrolkügelchen ist.

4. Einrichtung nach einem der Ansprüche 1 bis 3, wobei der Analyt ein Antikörper und der Ligand ein Antigen ist.

5. Einrichtung nach einem der Ansprüche 1 bis 3, wobei der Analyt ein Antigen und der Ligand ein Antikörper ist.

6. Einrichtung nach irgendeinem der vorstehenden Ansprüche, die in Fließrichtung hinter dem zweiten Abschnitt einen dritten Abschnitt aufweist, der mit dem zweiten Abschnitt in Kapillarströmungsverbindung steht.

7. Einrichtung nach irgendeinem der vorstehenden Ansprüche, wobei der zweite Abschnitt des Teststreifens Nitrocellulose aufweist.

8. Waschfreies einstufiges Verfahren zur Feststellung der Gegenwart oder Abwesenheit eines Analyten in

einer Flüssigkeitsprobe, das aus den folgenden Schritten besteht:
a) Zusetzen einer Flüssigkeitsprobe zum ersten Abschnitt der Einrichtung nach irgendeinem der vorstehenden Ansprüche;
b) Abwarten einer ausreichenden Zeit, damit die Flüssigkeit sich mit dem Tracer vermischen und das Gemisch aus Tracer und Flüssigkeit zum zweiten Abschnitt der Einrichtung nach Anspruch 1 fließen kann; und
c) Ablesen des Ergebnisses durch Nachweis der Gegenwart oder Abwesenheit des sichtbaren, aus Feststoffteilchen bestehenden nachweisbaren Markierungsstoffes im zweiten Abschnitt.

Revendications

1. Dispositif d'essai pour déterminer la présence ou l'absence d'un analyte dans un échantillon liquide comprenant:
a) une bande de test ayant au moins une première partie et une deuxième partie et étant disposées sur la bande dans le même plan d'une manière telle que la matière puisse s'écouler de la première partie vers la deuxième partie;
b) ladite première partie portant en elle de manière mobile un élément marqueur, dans laquelle ledit élément marqueur comprend un ligand spécifique pour l'analyte lorsque le dispositif est configuré pour un essai en sandwich et qui est l'analyte ou un analogue de celui-ci lorsque le dispositif est configuré pour un essai compétitif, conjugué à un marqueur particulaire non soluble et étant le site pour l'addition de l'échantillon;
c) ladite deuxième partie ayant, immobilisée en elle un liant qui est spécifique pour l'analyte lorsque ledit dispositif est configuré pour un essai en sandwich et qui est spécifique pour l'analyte et pour le ligand lorsque ledit dispositif est configuré pour un essai compétitif; le liant étant présent en une quantité telle que l'élément marqueur lié dans une telle partie soit visible.

2. Dispositif tel que revendiqué dans la revendication 1, dans lequel le marqueur particulaire détectable est un liposome coloré ou un grain de polymère coloré.

3. Dispositif tel que revendiqué dans la revendication 2, dans lequel le marqueur particulaire est un grain de polystyrène coloré.

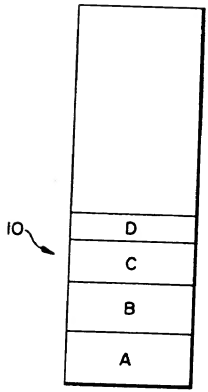
4. Dispositif tel que revendiqué dans l'une quelconque des revendications 1-3, dans lequel l'analyte est un anticorps et le ligand est un antigène.

5. Dispositif tel que revendiqué dans l'une quelconque des revendications 1 à 3, dans lequel l'analyte est un antigène et le ligand est un anticorps.

6. Dispositif tel que revendiqué dans l'une quelconque des revendications précédentes, qui comprend une troisième partie en aval de la deuxième partie et qui est en communication par écoulement capillaire avec ladite deuxième partie.

7. Dispositif tel que revendiqué dans l'une quelconque des revendications précédentes, dans lequel la deuxième partie de ladite bande de test comprend de la nitrocellulose.

8. Procédé en un seule étape sans lavage pour déterminer la présence ou l'absence d'un analyte dans un échantillon liquide constitué par les étapes de:
a) addition d'un échantillon liquide à la première partie du dispositif tel que revendiqué dans l'une quelconque des revendications précédentes;
b) attente d'un temps suffisant pour que le liquide se mélange avec l'élément marqueur et pour que le mélange de l'élément marqueur et de l'analyte s'écoule vers la deuxième partie du dispositif de la revendication 1; et
c) lecture du résultat en détectant la présence ou l'absence du marqueur particulaire détectable visible dans la seconde partie.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